UNITED STATES PATENT AND TRADEMARK OFFICE

COMMISSIONER FOR PATENTS
UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, DC 20231
www.uspto.gov

APPLICATION NO./ CONTROL NO.	FILING DATE	FIRST NAMED INVENTOR / PATENT IN REEXAMINATION	ATTORNEY DOCKET NO.
09/520,538	03/08/2000	WISE, A.	S-91,714

EXAMINER
STEADMAN

ART UNIT	PAPER
1652	4

DATE MAILED:

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents

This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 C.F.R. § 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 C.F.R. §§ 1.821-1.825 for the reason(s) set forth on the attached Notice To Comply With Requirements For Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures. Applicant must comply with the requirements of the sequence rules (37 CFR 1.821 - 1.825) before the application can be examined under 35 U.S.C. §§ 131 and 132.

APPLICANT IS GIVEN ONE MONTH FROM THE DATE OF THIS LETTER WITHIN WHICH TO COMPLY WITH THE SEQUENCE RULES, 37 C.F.R.. §§ 1.821-1.825. Failure to comply with these requirements will result in ABANDONMENT of the application under 37 C.F.R. § 1.821(g). Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 C.F.R. § 1.136. In no case may an applicant extend the period for response beyond the six month statutory period. Direct the response to the undersigned. Applicant is requested to return a copy of the attached Notice to Comply with the response.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David J. Steadman whose telephone number is (703) 308-3934. The examiner can normally be reached M-F from 8:00 am to 4:30 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy can be reached at (703) 308-3804. The fax number for the organization where this application or proceeding is assigned is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Office Action Summary	Application No. 09/520,538	Applicant(s) WISE ET AL.	
	Examiner David J. Steadman	Art Unit 1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-7 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-7 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☒ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

15)☒ Notice of References Cited (PTO-892)
16)☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
17)☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
18)☐ Interview Summary (PTO-413) Paper No(s). _____ .
19)☐ Notice of Informal Patent Application (PTO-152)
20)☐ Other:

DETAILED ACTION

Status of the Application

Claims 1-7 are pending in the application.

Oath/Declaration

1. The oath or declaration is defective. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02.

The oath or declaration is defective because: corrections made to the post office addresses of C. Kuske and T. Terwilliger have not been initialed as required.

Drawings

2. The drawings submitted with this application have not been reviewed by a draftsperson at this time. When formal drawings are submitted, the draftsperson will perform a review. Direct any inquiries concerning drawing review to the Drawing Review Branch (703) 305-8404.

Sequence Compliance

3. This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through 1.825; applicants' attention is directed to the final rulemaking notice published at 55 FR 18230 (May 1, 1990), and 1114 OG 29 (May 15, 1990). To be in compliance, applicants must provide an initial

computer readable form (CRF) copy of the "Sequence Listing", an initial paper copy of the "Sequence Listing", as well as an amendment directing its entry into the specification, and a statement that the content of the paper and CRF copies are the same and, where applicable, include no new matter as required by 37 C.F.R. 1.821(e) or 1.821(f) or 1.821(g) or 1.821(b) or 1.825(d). Applicant is requested to return a copy of the attached Notice to Comply with the response.

Specification/Informalities

4. The drawings are objected to by the Examiner because of the following informalities: the Y-axes of Figures 2-7 are not labeled.

Claim Objections

5. Claim 3 is objected to because of the recitation of "DmpR, MopR, PhhR, PhlR, XylR, and TbuT". Abbreviations, unless otherwise obvious should not be recited in the claims without at least once reciting the entire phrase(s) corresponding to the abbreviation(s). Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

6. Claims 1, 5, and 6 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

7. Claim 1 is confusing in that it is unclear as to what activities are in the "sensor domain" and which are in the "other domains". Clearly there must be at least the following "independent activities": an organic molecule binding domain, a DNA binding domain, and a transcriptional activation domain. It is suggested that Applicants clearly identify the "independent activities" of the "sensor domain" and the "other domains".

8. Claim 1 recites the limitation "A method for enhancing the response" (emphasis added). There is insufficient antecedent basis for this limitation in the claim. It is suggested that the term be replaced with, for example, "A method for enhancing a response".

9. The terms "enhanced" and "enhanced response" in claims 1, 5, and 6 are unclear absent a statement defining to what the response to the organic molecule is being compared. The terms "enhanced" and "enhanced response" in claims 1, 5, and 6 are relative terms and the claims should define and clearly state as to what the "enhanced response" to the organic molecules is being compared (i.e., enhanced in comparison to what level of response?).

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

10. Claims 1-7 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled

in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 1 (claims 2, 3, and 7 dependent thereon) and 4 (claims 5 and 6 dependent thereon) are directed to a method of enhancing the response of bacteria to organic molecules, said method utilizing a genus of: organic molecules, regulatory proteins, and genes encoding metabolic enzymes. The specification teaches only a single representative species of such organic molecules, i.e., phenols and six representative species of regulatory proteins, i.e., DmpR, MopR, PhhR, PhlR, XylR, and TbuT. Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the "functionality" of being organic molecules or regulatory proteins. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

11. Claims 1-7 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of enhancing the expression of a reporter gene under the control of a P_o promoter in *Pseudomonas* and *Acinetobacter* bacteria in response to the presence of phenols, said bacteria expressing a regulatory protein from the group consisting of DmpR, MopR, PhhR, PhlR, XylR, and TbuT with a sensor domain that binds phenols resulting in binding of the regulatory protein to a P_o promoter and activation of said reporter gene, said method comprising mutating the sensor domain of the regulatory protein by mutagenic PCR or gene shuffling, does not reasonably provide enablement for a method of enhancing any response of any bacteria to any organic molecules, said bacteria having any regulatory protein with any

sensor domain that binds to any cognate promoter sequence and activates expression of any genes encoding metabolic enzymes, said method comprising performing any modification to the sensor domain of the regulatory protein such that the response to the organic molecules is enhanced without altering the other domains, and optionally wherein the step of modifying is achieved by any mutation. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Claims 1 (claims 2, 3, and 7 dependent thereon) and 4 (claims 5 and 6 dependent thereon) are so broad as to encompass a method of enhancing any response of any bacteria to any organic molecules, said bacteria having any regulatory protein with any sensor domain that binds to any cognate promoter sequence and activates expression of any genes encoding metabolic enzymes, said method comprising performing any modification or any mutation to the sensor domain. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of bacteria and responses thereof, organic molecules, regulatory proteins, promoters, genes encoding metabolic enzymes, modifications, and mutations broadly encompassed by the claims. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. However, in this case the disclosure is limited to a method of enhancing the expression of a reporter gene under

the control of a P_o promoter in *Pseudomonas* and *Acinetobacter* bacteria in response to the presence of phenols, said bacteria expressing a regulatory protein from the group consisting of DmpR, MopR, PhhR, PhlR, XylR, and TbuT with a sensor domain that binds phenols resulting in binding of the regulatory protein to a P_o promoter and activation of said reporter gene, said method comprising mutating the sensor domain of the regulatory protein by mutagenic PCR or gene shuffling.

While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompass a method of enhancing any response of any bacteria to any organic molecules, said bacteria having any regulatory protein with a sensor domain that binds to any cognate promoter sequence and activates expression of any genes encoding metabolic enzymes, said method comprising performing any modification or any mutation to the sensor domain because the specification does not establish: (A) all conceivable responses of bacteria to an organic compound; (B) the general tolerance of any bacteria to any organic compound, as not all bacteria will elicit a response in the presence of any organic compound; (C) the general tolerance of any sensor domain of a regulatory protein to modification and extent of such tolerance; (D) a rational and

predictable scheme for performing any modifications or mutations of any sensor domain of a regulatory protein with an expectation of obtaining the desired biological function; (E) a reasonable expectation that any regulatory protein with a sensor domain can bind to any cognate promoter sequence and activate expression of any genes encoding metabolic enzymes; and (F) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including any number of amino acid modifications of any . The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

12. Claims 1-4 and 7 are rejected under 35 U.S.C. 102(b) as being anticipated by Shingler et al. (J Bacteriol 176:7550-7557). Claims 1-4 and 7 are drawn to a method of enhancing a response

of bacteria to organic molecules, said bacteria having a regulatory protein with discrete functional domains, one such domain being a sensor domain that binds a cognate promoter sequence and activates expression of genes encoding metabolic enzymes, said method comprising modifying the sensor domain of the regulatory protein such that the response to the organic molecules is enhanced without altering the other domains (claim 1), and optionally, wherein *Pseudomonas* and *Acinetobacter* bacteria are used (claim 2), and optionally, wherein the regulatory protein is selected from DmpR, MopR, PhhR, PhlR, XylR, and TbuT (claim 3), and optionally, wherein the sensor domain is mutated (claim 4), and optionally wherein the organic molecules are substitued phenols (claim 7). Shingler et al. teach that DmpR belongs to the NtrC family of transcriptional activators and shares significant sequence similarity with XylR, a *Pseudomonas* regulator of toluene and xylene catabolism (p 7550, Introduction, paragraph 1) and that DmpR responds to (methyl)phenols with the magnitude of transcriptional response differing depending on the position of the methyl substituent (p 7550, abstract). Shingler et al. also teach that DmpR and XylR are regulatory proteins composed of distinct functional domains (p 7550, Introduction) and that the A domains of these proteins bind aromatic compounds resulting in transcriptional activation (p 7556, paragraph 2). Shingler et al. further teach a method of mutating DmpR by chemical mutagenesis using ethyl methanesulfonate as mutagen (pp 7551-7552 under *Construction of P_o Km selection strain and isolation of DmpR specificity mutant*) to generate a mutant DmpR that, when expressed in *Pseudomonas putida* with a chromosomally inserted reporter gene (p 7552, under *Construction of P_o luxAB reporter strain and luciferase assays*), exhibits increased luciferase expression relative to wild-type DmpR in response to 4-methylphenol, 3,4-dimethylphenol, and 4- ethylphenol (p 7554, Fig 3) and that sequencing the

gene encoding the DmpR mutant revealed a mutation at codon 135 (p 7554, under *Genetic selection of an effector specificity mutant, DmpR-E135K*) of the A domain of DmpR (amino acids 1-211; p 7556, paragraph 2). Shingler et al. also teach that a comparison of the responses of the wild-type and mutant DmpR to various phenolic derivatives suggests that, in addition to the increased responses of the mutant to 4-methylphenol, 3,4-dimethylphenol, and 4-ethylphenol, the mutant DmpR mediates responses to phenol, 2-methylphenol, and 3-methylphenol to similar extents as wild-type DmpR (p 7554, under *Effector profile comparison of DmpR$^+$ and DmpR-E135K*), suggesting that the mutant DmpR response to 4-methylphenol, 3,4-dimethylphenol, and 4-ethylphenol is enhanced relative to wild-type DmpR without altering the function of the other (DNA binding and transactivation) domains. This anticipates claims 1-4 and 7 as written.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

13. The bacteria and regulator proteins of claims 2 and 3 not addressed in the 35 U.S.C. 102(b) rejection above are rejected under 35 U.S.C. 103(a) as being unpatentable over Shingler et al. in view of any of Willardson et al. (Appl Environ Microbiol 64:1006-1012), Schirmer et al. (J Bacteriol 179:1329-1336), Ng et al. (J Bacteriol 177:1485-1490), Burchhardt et al. (Mol Gen Genet 254:539-547), or Byrne et al. (J Bacteriol 178:6327-6337). These embodiments of claims 2 and 3 are drawn to a method of enhancing the response of bacteria to organic molecules by

mutating the sensor domain of a regulatory protein in *Acinetobacter* (claim 2) or wherein the regulatory protein is selected from MopR, PhhR, PhlR, and TbuT (claim 3).

In addition to the teachings described above, Shingler et al. teach that DmpR is activated by a wide range of phenolic compounds, however, the response to *para*-substituted phenolic compounds (i.e., 4-methylphenol and 3,4-methylphenol) is relatively poor (p 7550, Introduction). Shingler et al. further teach "the amino acid changes in the DmpR and XylR effector specificity mutants are Glu-to-Lys changes at residues 135 and 172, respectively, and in each case the regulators have been shown to gain the ability to recognize a novel effector compound" (p 7556, left column, bottom). Shingler et al. also teach that their data, in combination with previoius teachings, "suggest that activation of DmpR and XylR is mediated by aromatic effector binding to the A domains of these regulators" (p 7556, left column, bottom). Shingler et al. do not teach a method of enhancing the response of bacteria to organic molecules by mutating the sensor domain of a regulatory protein expressed in *Acinetobacter* or wherein the regulatory protein is selected from MopR, PhhR, PhlR, and TbuT.

Willardson et al. teach a biosensor using *Escherichia coli* expressing XylR that responds to toluene and derivatives thereof by luminescence proportional to the concentration of toluene or derivatives thereof present in a medium. Willardson et al. further teach "the development of this biosensor for toluene and its derivative compounds demonstrates the feasibility of constructing similar biosensors with specificity for other organic contaminants by using their corresponding transcriptional activators" (p 1011, bottom – 1012, top). Willardson et al. also suggest using other bacterial strains as biosensors (p 1012, top).

Schirmer et al. teach MopR is regulator protein of phenol metabolism in *Acetobacter calcoaceticus* (p 1329, Introduction) and teach characterization of MopR response to various effector compounds (p 1332, Fig 3), the results of which suggest that MopR exhibits a response to 3,4-dichlorophenol, while DmpR does not respond to the same compound (p 1334, right column). Schirmer et al further teach the A domain of MopR consists of residues 1-150 (p 1333, left column).

Ng et al. teach PhhR is regulator protein of phenol and monomethylated phenol metabolism in *Pseudomonas putida* (p 1485, Introduction) and teach characterization of PhhR response to various effector compounds (p 1489, Fig 4). Ng et al further teach the A domain of PhhR consists of residues 1-210 (p 1487, right column).

Burchhardt et al. teach PhlR is a regulator protein of phenol metabolism in *Pseudomonas putida* (p 539, Abstract) and disclose the nucleotide sequence and a method of expressing PhlR in bacteria (p 540-541 under Materials and Methods). Burchhardt et al. also teach that PhlR shares relatively high homology with XylR, PhhR, and DmpR (p 542, left column).

Byrne et al. teach TbuT is a regulator protein of toluene metabolism in *Burkholderia pickettii* (p 6327, Introduction) and teach characterization of TbuT response to various effector compounds (p 6333, Fig 6), the results of which suggest that TbuT exhibits a response to trichloroethylene (p 1334, right column), and disclose that this is the first regulator protein that responds to trichloroethylene (p 6336, right column). Byrne et al further teach the A domain of TbuT consists of residues 1-238 (p 6333, Fig 5).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Shingler et al. with any of Willardson et al., Schirmer et al., Ng et al., Burchhardt et

al., and Byrne et al. for a method of enhancing the response of bacteria to organic molecules by mutating the sensor domain of a regulatory protein expressed in *Acinetobacter* or wherein the regulatory protein is selected from MopR, PhhR, PhlR, and TbuT. One would have been motivated to mutate the sensor domain of other regulator proteins and use other bacteria for regulator protein expression because of the teachings of Shingler et al. who taught that the binding specificity of regulatory proteins can be broadened by mutating the sensor domain and Willardson et al. who taught other biosensors can be generated with specificity for other organic contaminants under varying conditions. One would have a reasonable expectation of success for a method of enhancing the response of bacteria to organic molecules by mutating the sensor domain of a regulatory protein expressed in *Acinetobacter* or wherein the regulatory protein is selected from MopR, PhhR, PhlR, and TbuT because of the results of Shingler et al., Willardson et al., Schirmer et al., Ng et al., Burchhardt et al., and Byrne et al. Therefore, claims 2 and 3, drawn to a method of enhancing the response of bacteria to organic molecules by mutating the sensor domain of a regulatory protein expressed in *Acinetobacter* or wherein the regulatory protein is selected from MopR, PhhR, PhlR, and TbuT would have been obvious to one of ordinary skill in the art.

14. Claims 5 and 6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Shingler et al. in view of Willardson et al. and either of Cadwell et al. ("Mutagenic PCR" pp 583-589 in "PCR Primer, A Laboratory Manual", Cold Spring Harbor Laboratory Press, 1995) or Stemmer (Nature 370:389-391). Claims 5 and 6 are drawn to a method of enhancing the response of bacteria to organic molecules by mutating the sensor domain of a regulatory protein by mutagenic PCR (claim 5) or gene shuffling (claim 6).

Shingler et al. disclose the teachings described above. Shingler et al. do not teach methods of enhancing bacterial response to organic molecules by mutating the gene encoding DmpR using mutagenic PCR or gene shuffling.

Willardson et al. disclose the teachings described above.

Cadwell et al. teach a mutagenic PCR method of randomly mutating a nucleic acid in order to generate a library of mutant nucleic acids (p 584 under *Protocol*). Cadwell et al. further teach that using these mutants, one can apply a screening method to isolate individual clones that exhibit a particular property. (p 583, Introduction, paragraph 2).

Stemmer et al. teach a method of *in vitro* homologous recombination of pools of selected mutant genes by random fragmentation and PCR reassembly, i.e., gene shuffling (p 390, Fig 1) and teach that one would use gene shuffling over mutagenic PCR because mutagenic PCR is not combinatorial and thus, is more limited in the number of possible mutations (p 389, abstract and p 390, right column).

It would have been obvious to one of ordinary skill in the art at the time of the invention to mutate only the sensor domain, i.e., the A domain as described by Shingler et al. because one of ordinary skill would have recognized that, in order to broaden the binding specificity of the sensor domain, one need mutate the only the protein domain responsible for binding the effector compound, i.e., the sensor domain and not the DNA binding or transactivation domains of DmpR and XylR. Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Shingler et al., Willardson et al. and either of Cadwell et al. or Stemmer for a method of mutating the sensor domain of a regulatory protein by mutagenic PCR or gene shuffling. One would have been motivated to alter the method of mutating the sensor domain as

taught by Shingler et al. by mutating the sensor domain of a regulatory protein by mutagenic PCR or gene shuffling because of the teachings of Cadwell et al. who taught a library of mutant nucleic acids can be generated by mutagenic PCR and that using these mutants, one can isolate individual clones that exhibit a particular property or Stemmer who taught the limitation of mutagenic PCR. One would have a reasonable expectation of success for a method of enhancing the response of bacteria to organic molecules by mutating the sensor domain of a regulatory protein by mutagenic PCR or gene shuffling because of the results of Shingler et al. Therefore, claims 5 and 6, drawn to a method of enhancing the response of bacteria to organic molecules by mutating the sensor domain of a regulatory protein by mutagenic PCR or gene shuffling would have been obvious to one of ordinary skill in the art.

15. No claim is in condition for allowance.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David Steadman, whose telephone number is (703) 308-3934. The examiner can normally be reached Monday-Friday from 8:00 am to 4:30 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, can be reached at (703) 308-3804. The FAX number for this Art Unit is (703) 308-4242. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Art Unit receptionist whose telephone number is (703) 308-0196.

David J. Steadman

Rebecca Prouty

REBECCA E. PROUTY
PRIMARY EXAMINER
GROUP ~~1800~~ 1600